# Effects of dietary supplementation of L-methionine vs. DL-methionine on performance, plasma concentrations of free amino acids and other metabolites, and myogenesis gene expression in young growing pigs

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**ABSTRACT:** Methionine (Met), the second or third limiting amino acid (AA) in typical swine diets, plays important roles in promoting swine health and growth, especially, muscle growth. Whereas DL-Met products have been used in swine industry for many years, L-Met products have been developed recently. This research was conducted to study the effects of supplemental L-Met or DL-Met on nutrient metabolism, muscle gene expression, and growth performance of pigs. Twenty crossbred young barrows (initial body weight [BW] 21.2 ± 2.7 kg) were randomly assigned to 20 individual pens and two dietary treatments according to a completely randomized design with pigs serving as the experiment unit ($n = 10$). Two corn and soybean meal-based diets (diets 1 and 2) were formulated to meet or exceed the recommended requirements for energy, AA, and other nutrients (NRC. 2012. Nutrient requirements of swine, 11th ed. Washington, DC: The National Academies Press; AMINODat 5.0). Crystalline L-Met and DL-Met were supplemented to diets 1 and 2 (both at 0.13%, as-fed basis), respectively. After 4 wk of an ad libitum feeding trial, BW and feed intake were measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Blood samples were collected from the jugular vein for analyses of plasma AA and metabolite concentrations. The longissimus dorsi muscle samples were collected for analysis of myogenesis gene expression. Data were analyzed using Student's *t*-test. There were no differences ($P = 0.56$ to 0.94) in ADG, ADFI, or G:F between pigs fed the two experimental diets and no differences between diets were observed in plasma free AA concentrations. No differences were observed between pigs fed the two diets in expression of mRNA for eight myogenesis-related genes, which were myogenic differentiation 1, myogenin, myogenic factors 5, muscle regulatory factor 4 (a.k.a. myogenic factors 6), and myocyte enhancer factors 2A, 2B, 2C, and 2D. In conclusion, results of this experiment indicate that the bioefficacy of L-Met is not different from that of DL-Met, which is likely because of an efficient conversion of D-Met to L-Met by pigs.

**Key words:** DL-methionine, growth performance, L-methionine, muscle gene expression, pig, plasma amino acid and metabolite



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## INTRODUCTION

The primary tissue component of pork is skeletal muscle and, thus, knowledge concerning the

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growth and development of muscle in pigs is crucial for the industry from either an economic or a technical standpoint (Liao et al., 2015). Proteins, the major chemical component of muscle, are synthesized from amino acids (AA), which originate from dietary proteins including supplemented crystalline AA (O'Connor et al., 2003; Li et al., 2016). Among the 20 proteinogenic AA in nature, 10 are dietarily indispensable because pigs cannot de novo synthesize them or cannot synthesize enough to meet their metabolic requirements (NRC, 2012). Methionine (Met) is typically the second or third limiting indispensable AA in grain-based swine diets.

In addition to serving as a building block for protein synthesis, Met plays important roles in exerting numerous metabolic and physiological functions in pigs, including functioning as a major methyl donor, as an important source of sulfur, as an endogenous antioxidant, and as precursors of many bioactive compounds (Zhai et al., 2012; Shen et al., 2014). Dietary supplementation of Met can increase nitrogen (N) retention and muscle protein accretion in pigs via increasing protein synthesis and decreasing protein degradation and, thus, improve growth performance (Chung and Baker, 1992a; Zimmermann et al., 2005; Kim et al., 2006; Wen et al., 2014; Kong et al., 2016a, 2016b).

Dietary supplementation of Met has been practiced for many years in the swine industry. Feed-grade crystalline DL-Met has been a conventional form of commercial Met products, which consists of 50% D-Met and 50% L-Met. Recently, another form of feed-grade Met, L-Met, has become available on the market. The L-Met products are produced either from chemical synthesis or from fermentation of plant-based raw materials (Willke, 2014). Research has been conducted to investigate the nutritional value of L-Met in pigs (Shen et al., 2014; Kong et al., 2016a, 2016b; Tian et al., 2016), but several aspects of the mechanism by which L-Met affects muscle growth have not been elucidated. This study was conducted to investigate the effects of L-Met vs. DL-Met on growth performance, plasma concentrations of 22 free AAs and six representative metabolites, as well as the expression of eight genes related to myogenesis in growing pigs. The overall hypothesis of this study was that the nutritional bioefficacy of L-Met is higher than that of DL-Met, which would be reflected in the parameters measured in the study.

## MATERIALS AND METHODS

All experimental procedures involving caring, handling, and treatment of pigs were approved by the Mississippi State University Institutional Animal Care and Use Committee.

### *Animal Feeding Trial*

A total of 20 crossbred (Large White × Landrace) young growing barrows (around 5 wk of age) were purchased from a local commercial farm and transferred to an environmentally controlled swine barn at the Leveck Animal Research Center of Mississippi State University. After arrival, pigs were assigned into five feeding pens and fed a commercial nursery diet until their body weight (BW) reached 21.2 ± 2.7 kg, during which period pigs were allowed ad libitum access to the diet and water. Pigs were then randomly assigned to 20 individual pens, and allotted to two dietary treatments according to a completely randomized experimental design with pigs serving as the experimental unit.

On the basis of analyzed AA contents in the major feed ingredients used, two corn and soybean meal-based diets (diets 1 and 2) were formulated to meet the NRC (2012) requirements for energy, crude protein (CP), minerals, and vitamins. For indispensable AA, the requirement standards of AMINODat 5.0 (Platinum Version, 2016. Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany) were followed. Crystalline L-Met and DL-Met were supplemented to diets 1 and 2 (both at 0.13%, as-fed basis), respectively, to meet pigs' requirement for Met (Table 1). Supplemental DL-Met (MetAMINO) and L-Met products were obtained from Evonik Nutrition & Care GmbH. To confirm the contents of major nutrients, representative samples of the two diets were submitted to the Essig Animal Nutrition Laboratory at Mississippi State University for energy and proximate analyses, and to an Evonik's laboratory in Hanau-Wolfgang, Germany for AA analysis. The analyzed composition of selected nutrients contained in the two diets is shown in Table 2.

During the 4-wk feeding trial, pigs were allowed ad libitum access to experimental diets, and water was available at all times. All feeders and waterers were checked at least three times daily (0600 to 2200 h) to ensure proper function of the facilities and normal behavior of animals. Orts and refusal feed were collected and returned to the feeders immediately or reserved and weighed for feed intake calculation. Individual pig BW were recorded at the beginning and the end of the 4-wk experiment. Daily feed allotments were recorded as well, and at the conclusion of the experiment, data were summarized to calculate average daily gain (ADG),

**Table 1.** Composition of the experimental diets fed to the growing pigs (as-fed basis)[a]

| | Dietary treatment | |
|---|---|---|
| Item | Diet 1 | Diet 2 |
| Ingredient, % | | |
| Corn | 74.88 | 74.88 |
| Soybean meal | 20.00 | 20.00 |
| Poultry fat | 1.27 | 1.27 |
| L-Lysine HCl, 78.8% | 0.58 | 0.58 |
| Methionine[b], 99% | 0.13 | 0.13 |
| L-Threonine, 98.5% | 0.24 | 0.24 |
| L-Tryptophan, 98% | 0.07 | 0.07 |
| L-Isoleucine, 96% | 0.10 | 0.10 |
| L-Valine, 96.5% | 0.18 | 0.18 |
| L-Cysteine HCl, 99.7% | 0.09 | 0.09 |
| Limestone | 0.81 | 0.81 |
| Dicalcium phosphate | 1.30 | 1.30 |
| Salt | 0.18 | 0.18 |
| Mineral premix[c] | 0.10 | 0.10 |
| Vitamin premix[c] | 0.07 | 0.07 |
| Total | 100.00 | 100.00 |
| Major nutrients, calculated[d] | | |
| Dry matter, % | 87.86 | 87.86 |
| Net energy (kcal/kg) | 2,440 | 2,440 |
| Crude fat | 4.17 | 4.17 |
| Crude fiber | 2.30 | 2.30 |
| Ash | 2.14 | 2.14 |
| SID CP, % | 14.14 | 14.14 |
| SID lysine, % | 1.08 | 1.08 |
| SID methionine, % | 0.37 | 0.37 |
| SID methionine + cysteine, % | 0.67 | 0.67 |
| SID threonine, % | 0.70 | 0.70 |
| SID tryptophan, % | 0.22 | 0.22 |
| SID valine, % | 0.80 | 0.80 |
| SID isoleucine, % | 0.66 | 0.66 |
| Total calcium, % | 0.66 | 0.66 |
| STTD phosphorus, % | 0.37 | 0.37 |
| Crude fiber, % | 2.30 | 2.30 |
| Ash, % | 2.14 | 2.14 |

[a]L-Lysine HCl (containing 78.8% L-lysine) and L-threonine were purchased from Archer Daniels Midland Co (Quincy, IL). L-Tryptophan and L-valine were donated from Ajinomoto Heartland, Inc (Chicago, IL). L-Cysteine HCl (containing 76.9% L-cysteine) was purchased from Wuhan Grand Hoyo Co, Ltd (Wuhan, Hubei, China).

[b]L-methionine and DL-methionine (MetAMINO) were obtained from Evonik Nutrition & Care GmbH (Hanau-Wolfgang, Germany) and used in diets 1 and 2, respectively.

[c]Mineral premix (No. NB-8534) and vitamin premix (No. NB-6508A) were donated from Nutra Blend, LLC (Neosho, MO). The calculated mineral and vitamin contents in both diets were (per kg of diet): Na, 1.0 g; Cl, 2.8 g; K, 6.1 g; Mg, 1.4 g; S, 1.5 g; Cu, 16.7 mg; Fe, 172.0 mg; I, 0.20 mg; Mn, 37.9 mg; Zn, 132.1 mg, Se, 0.28 mg; vitamin A, 3,081 IU; vitamin $D_3$, 385 IU; vitamin E, 29.5 IU; vitamin K, 1.23 mg; vitamin $B_1$, 2.29 mg; vitamin $B_2$, 3.65 mg; niacin, 36.2 mg; vitamin $B_3$, 13.3 mg; vitamin $B_6$, 5.02 mg; biotin, 0.10 mg; folacin, 0.39 mg; vitamin $B_{12}$, 10.8 µg, and choline, 1.39 mg. [d]

SID = standardized ileal digestible. STTD = standardized total tract digestible.

**Table 2.** The analyzed nutrient composition (%, or as indicated) of the two experimental diets fed to the growing pigs (as-fed basis)[a]

| | Dietary treatment | |
|---|---|---|
| Nutrient and energy[b] | Diet 1 | Diet 2 |
| Proximate analysis | | |
| Dry matter | 88.8 | 88.7 |
| Gross energy, kcal/kg | 3,969 | 3,944 |
| CP | 16.28 | 16.72 |
| Crude fat | 3.28 | 2.94 |
| Crude fiber | 2.03 | 1.87 |
| Ash | 4.13 | 4.44 |
| Dietary total AA | | |
| Lysine | 1.23 | 1.28 |
| Methionine | 0.41 | 0.41 |
| Cysteine | 0.33 | 0.33 |
| Methionine + cysteine | 0.74 | 0.75 |
| Threonine | 0.84 | 0.82 |
| Tryptophan | 0.26 | 0.25 |
| Arginine | 1.02 | 1.01 |
| Histidine | 0.43 | 0.43 |
| Leucine | 1.43 | 1.41 |
| Isoleucine | 0.74 | 0.74 |
| Valine | 0.94 | 0.95 |
| Phenylalanine | 0.77 | 0.76 |
| Proline | 1.00 | 1.01 |
| Aspartic acid | 1.56 | 1.52 |
| Glutamic acid | 2.84 | 2.79 |
| Serine | 0.80 | 0.78 |
| Alanine | 0.85 | 0.84 |
| Glycine | 0.66 | 0.66 |
| Dietary free AA | | |
| Lysine | 0.43 | 0.48 |
| Methionine | 0.14 | 0.16 |
| Threonine | 0.25 | 0.25 |
| Valine | 0.20 | 0.22 |
| Isoleucine | 0.10 | 0.10 |

[a]Diet 1 = a L-methionine supplemented diet; diet 2 = a DL-methionine supplemented diet.

[b]Proximate and energy analyses were conducted at the Essig Animal Nutrition Laboratory, Mississippi State University (Starkville, MS). AA analysis was conducted at the analytical laboratory of Evonik Nutrition & Care GmbH (Hanau-Wolfgang, Germany).

average daily feed intake (ADFI), and gain-to-feed ratio (G:F) for each pen and each treatment group.

### *Sample Collection and Analyses*

At the beginning and also at the end of the 4-wk experiment, blood samples were collected via jugular venipuncture (approximately 10 mL/pig) in early morning (between 0600 and 0800 h). Immediately before bleeding, feed in all feeders was removed. Collected blood samples were placed on

ice until plasma was separated within 30 min by centrifugation of the samples at 800 × *g* and 4 °C for 16 min. Blood plasma samples in 500-μL aliquots were then stored at –80 °C until laboratory analyses of nutrient metabolites and free AA.

After blood collection, a muscle sample (about 200 mg) was collected from the middle portion of longissimus dorsi muscle of each pig using our standard aseptic biopsy protocol (Burnett et al., 2016). All muscle samples collected were snap frozen in liquid N, and then transferred to a –80 °C freezer for storage until analyses.

For determination of plasma metabolites, batch analysis was performed using the automated ACE Alera Clinical Chemistry System (Alfa Wassermann, West Caldwell, NJ) with six respective ACE reagents (Alfa Wassermann) for glucose, total protein, albumin, urea N, total cholesterol, and total triglycerides. Determination of these metabolites involved enzymatic reactions with appropriate enzymes, followed by bichromatic measurements of respective reaction products at different wavelengths to determine the concentrations. Briefly, the glucose assay was conducted using the hexokinase method (Todd et al., 1979). Urea N concentration was determined using the Urease-GLDH method (Tietz et al., 1995). The cholesterol assay was conducted using a hydrolysis method involving cholesterol esterase, cholesterol oxidase, and peroxidase (Artiss and Zak, 1997). The triglyceride assay was also conducted with an enzymatic method involving lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase (Kalia and Pundir, 2004). The assay of albumin concentration involved specific binding between bromocresol green and albumin (Tietz et al., 1995). Likewise, the assay of total protein involved a reaction of cupric ions with peptide bonds under alkaline conditions (Tietz et al., 1995).

The concentrations of plasma free AA were determined using high-performance liquid chromatography (HPLC) methods (Liao et al., 2005; Wu and Meininger, 2008; Dai et al. 2014). Briefly, after a pre-column derivatization of plasma AA with *o*-phthaldialdehyde, samples were separated on a Supelco 3-μm reversed-phase C18 column (4.6 × 150 mm, i.d.) guarded by a Supelco 40-μm reversed-phase C18 column (4.6 × 50 mm, i.d.). The HPLC mobile phase consisted of solvent A (0.1 M sodium acetate/0.5% tetrahydrofuran/9% methanol; pH 7.2) and solvent B (methanol), with a combined total flow rate of 1.1 mL/min. A gradient program with a total running time of 49 min was developed for satisfactory separation of AA.

### *Gene Expression Analyses*

Total RNA was extracted from approximately 50 mg of muscle sample per animal using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's instructions. Briefly, frozen tissue was homogenized in a 15-mL polypropylene centrifuge tube using a Polytron mixer (0.5 mL TRIzol per 50 mg tissue), and the homogenate transferred to a 1.5-mL micro-centrifuge tube. Chloroform (400 μL/tube) was used to separate RNA from DNA and proteins, and then the total RNA was precipitated with isopropyl alcohol (at 1:1 ratio) and washed with 750 μL of 75% ethanol. The resulted RNA was air-dried, dissolved in 60 μL RNase-free water, and stored at –80 °C in a freezer. The purity and concentration of the RNA samples were measured using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

First-strand cDNA were reverse-transcribed from 1 μg of total RNA by using QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA). The semi-quantitative polymerase chain reaction (PCR) analysis was performed with the Rotor-Gene SYBR Green PCR Kit using the Rotor-Gene Q System (Qiagen), followed by melting curve analysis to verify the specificity and identity of the PCR products. The thermal cycling parameters were 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 10 s. Primers for the selected genes were designed by using PrimerQuest Tool (Integrated DNA Technologies, Coralville, IA). The sequences of the designed primers and the relevant information associated with these primers are shown in Table 3. Also shown in Table 3 is the endogenous control gene, *Hprft1* (hypoxanthine phosphoribosyl transferase 1), used for normalization of any variation during the sample preparation (Nygard et al., 2007).

The comparative $\Delta\Delta C_T$ method was used for mRNA quantity calculation. Briefly, the raw quantity of a given gene was normalized against the raw quantity of *Hprft1* reference gene of a given sample obtained from the Rotor-Gene Q System, and then the normalized level of the given gene of each sample was expressed as a quantity relative to the mean of the normalized quantities of the given gene of the diet 2 samples.

### *Statistical Analysis*

Data were subjected to statistical analysis with Student's *t*-test by using the SAS software (version

**Table 3.** Real-time PCR primers for the selected genes

| Gene name | Gene symbol | RefSeq ID[a] | Primer sequence[b] | Amplicon size (bp) |
|---|---|---|---|---|
| Myogenic differentiation 1 | *MYOD1* | NM_001002824 | CCTAAAGCCCGAGGAACACT<br>TAGTGGTCTTGCGTTTGCAC | 152 |
| Myogenin | *MYOG* | NM_001278775 | GACGAGTTTGAGCCACGAGT<br>ATTTCCTCTTGCACGCTTTG | 148 |
| Myogenic factor 5 | *MYF5* | NM_001012406 | AGTGCCCCTGGAAGATAAGG<br>GGCCTCATTCACCTTCTTGA | 160 |
| Myogenic regulatory factor 4 | *MRF4* | NM_001244672 | ACAAAATGCAGGAGCTAGGC<br>CTCCTTCCTTGGCAGTCATC | 150 |
| Myocyte enhancer factor 2A | *MEF2A* | NM_001097421 | GGTGCTGACGGGTACAACTT<br>AATTCCTGCATTCGTTCCTG | 149 |
| Myocyte enhancer factor 2B | *MEF2B* | XM_003362215 | GCTCTGCGACTGTGAGATTG<br>GTCTCGAGGATGTCGGTGTT | 150 |
| Myocyte enhancer factor 2C | *MEF2C* | NM_001044540 | CCAGGCAGCAAGAATACGAT<br>TTGTTGAAATGGCTGATGGA | 148 |
| Myocyte enhancer factor 2D, transcript variant X1 | *MEF2D* | XM_003125698 | AAGAGGAAGTTCGGGCTGAT<br>CTCCGTGTACTTGAGCAGCA | 147 |
| Hypoxanthine phosphoribosyl transferase 1 | *HPRFT1* | NM_001032376 | GCTATGCCCTTGACTACAATGA<br>TTGAACTCTCCTCTTAGGCTTTG | 102 |

[a]All these RefSeq sequences are porcine (*Sus scrofa*) specific from NCBI (http://www.ncbi.nlm.nih.gov/refseq/rsg/).

[b]Forward and reverse primers for each of the nine genes are presented in the 5′ to 3′ direction.

9.4; SAS Institute Inc, Cary, NC). A $P$ value less than 0.05 was considered as a significant difference between treatment means, and a $P$ value between 0.05 and 0.10 as a tendency. Each value of the measurements is presented as mean ± SD.

## RESULTS

As shown in Table 2, the analyzed contents of nutrients, especially, the CP and AA, in the two experimental diets were close to the calculated values (Table 1). The initial BW were not different ($P$ = 0.94) between the two groups of pigs (Table 4). The final BW of pigs fed the 2 diets were not different and overall ADG, ADFI, and G:F were also not different.

At the beginning of the experiment, there was no difference in plasma concentrations of urea N, total protein, albumin, glucose, total cholesterol, and triglycerides between the pigs fed diets 1 and 2 (Table 5). After feeding the experimental diets for 4 wk, there were no differences in the plasma concentrations of urea N, albumin, glucose, total cholesterol, and triglycerides ($P$ = 0.38 to 0.93), although the plasma total protein concentration of the pigs fed diet 2 tended to be higher than that of the pigs fed diet 1 ($P$ = 0.10). The overall results indicated that the pigs fed the L-Met supplemented diet had plasma concentrations of these six metabolites that were not different from that of pigs fed the DL-Met supplemented diet.

At the beginning of the experiment, there were no differences ($P$ = 0.12 to 0.98) in plasma concentrations of AA between the two groups of pigs (Table 6). At the end of the experiment, plasma concentrations of all these AAs were still not different between the two groups of pigs (Table 7; $P$ = 0.20 to 0.99), which indicated that the AA metabolism and protein synthesis were not altered by the Met forms.

Before the feeding trial, the mRNA expression levels of the eight myogenesis-related genes were similar ($P$ = 0.14 to 1.00) between the pigs fed diets 1 and 2 (Table 8). After the 4-wk feeding trial, there were still no differences ($P$ = 0.18 to 0.94) between these two groups of pigs in the mRNA expression levels of these eight genes (Table 8). These results indicated that the dietary supplementation of L-Met vs. DL-Met do not affect the expression of these genes differently.

## DISCUSSION

### *Growth Performance*

As previously reported, L-Met can be directly used by animals for metabolism and protein synthesis (Dibner and Ivey, 1992; Stoll et al., 1998; Martín-Venegas et al., 2006; Shen et al., 2014; Willke, 2014; Kong et al., 2016a; Tian et al., 2016). D-Met, however, needs to be converted to L-Met via a biochemical pathway and then become bioavailable for metabolic processes including muscle growth in pigs (Baker, 2006). The efficiency of metabolic utilization of exogenous D-Met depends on how it

**Table 4.** Growth performance of the pigs fed a L- vs. DL-methionine supplemented diet[a]

| Item | Dietary treatment | | *P* value[b] |
|---|---|---|---|
| | Diet 1 | Diet 2 | |
| Initial BW, kg | 21.3 ± 2.68 | 21.2 ± 2.80 | 0.942 |
| Final BW, kg | 48.0 ± 5.71 | 48.2 ± 5.35 | 0.942 |
| ADG, kg/d | 0.95 ± 0.12 | 0.96 ± 0.13 | 0.868 |
| ADFI, kg/d | 1.71 ± 0.23 | 1.75 ± 0.24 | 0.691 |
| G:F | 0.56 ± 0.02 | 0.55 ± 0.03 | 0.560 |

[a]Diet 1 = a L-methionine supplemented diet; diet 2 = a DL-methionine supplemented diet. The calculated dietary standardized ileal digestible methionine contents in both diets 1 and 2 were 0.37% (as-fed basis). Each value is a mean ± SD ($n$ = 10).

[b]*P* values were obtained from Student's *t*-test.

**Table 5.** The concentrations of selected metabolites in the blood plasma of growing pigs before and after the 4-wk feeding trial[a]

| Metabolites | Dietary treatment | | *P* value[b] |
|---|---|---|---|
| | Diet 1 | Diet 2 | |
| Before the feeding trial | | | |
| Urea N, mg/dL | 9.00 ± 1.82 | 8.00 ± 1.25 | 0.170 |
| Total protein, g/dL | 4.54 ± 0.49 | 4.78 ± 0.42 | 0.258 |
| Albumin, g/dL | 2.34 ± 0.33 | 2.34 ± 0.28 | 1.000 |
| Glucose, mg/dL | 118.3 ± 17.86 | 127.0 ± 15.10 | 0.255 |
| Total cholesterol, mg/dL | 76.1 ± 14.62 | 77.9 ± 9.72 | 0.750 |
| Triglycerides, mg/dL | 40.3 ± 16.39 | 41.9 ± 12.16 | 0.807 |
| After the feeding trial | | | |
| Urea N, mg/dL | 6.30 ± 2.63 | 6.40 ± 2.59 | 0.933 |
| Total protein, g/dL | 5.46 ± 0.32 | 5.70 ± 0.3 | 0.096 |
| Albumin, g/dL | 3.37 ± 0.17 | 3.46 ± 0.26 | 0.376 |
| Glucose, mg/dL | 114.5 ± 11.8 | 111.4 ± 7.68 | 0.496 |
| Total cholesterol, mg/dL | 74.1 ± 12.11 | 74.6 ± 11.45 | 0.926 |
| Triglycerides, mg/dL | 47.5 ± 10.69 | 51.3 ± 22.11 | 0.631 |

[a]Diet 1 = a L-methionine supplemented diet; diet 2 = a DL-methionine supplemented diet. The calculated dietary standardized ileal digestible methionine contents in both diets 1 and 2 were 0.37% (as-fed basis).

[b]*P* values were obtained from Student's *t*-test.

can be efficiently transformed to L-Met. Thus, for a DL-Met product, composed of 50% D-Met and 50% L-Met, the efficacy of its utilization relies on the efficiency of the metabolic conversion of D-Met to L-Met. All D-Met can be converted to L-Met by pigs (Wretlind and Rose, 1950; Cho and Stegink, 1979; Sugiyama and Muramatsu, 1987; Hasegawa et al., 2005; Kong et al., 2016b). Indeed, the capacity of the rate-limiting enzyme, i.e., D-amino acid oxidase (D-AAOX), to convert D-Met to L-Met is not limiting in pigs (Fang et al., 2010) and poultry (Brachet and Puigserver, 1992) due to the existence of substantial D-AAOX activity in different tissues including liver and kidney (the major conversion sites), and also in the gastrointestinal tract (stomach, duodenum, jejunum, and ileum).

Although a tendency of increasing ADG in weaned pigs fed diets containing L-Met instead of DL-Met was reported (Reifsnyder et al., 1984; Shen et al., 2014), several other studies found that the pigs fed with L-Met supplemented diets had no difference in growth performance when compared with pigs fed DL-Met supplemented diets (Chung and Baker, 1992b; Chen et al., 2013; van Milgen et al., 2013; Kong et al., 2016a, 2016b). Htoo and Morales (2016) also reported that the growth performance of weaned pigs fed diets supplemented with same inclusion levels of DL-Met and L-Met were not different and, thus estimated a 100% relative bioavailability of L-Met using a slope-ratio regression method. Using N-balance, Tian et al. (2016) demonstrated that dietary supplementation with L-Met or DL-Met (at the same inclusion level) to a Met deficient diet improved N retention and decreased fecal N excretion, but there were no differences between L-Met and DL-Met supplemented diets in terms of N retention or fecal N excretion. These results suggested that L-Met and DL-Met as Met sources are equally bioavailable for pigs, and results of the present experiment are consistent with these previous data.

### *Plasma Metabolites and Free Amino Acids*

Several plasma parameters, such as plasma urea N, total protein, albumin, total cholesterol, triglycerides, and glucose, are indicative of the nutritional status of animals (Wen et al., 2014; Regmi et al., 2018). It is worth mentioning that the values for these parameters obtained in this study are similar to those obtained by Hu et al. (2015), who also measured these parameters in growing pigs.

As an indicator of AA utilization by pigs, plasma concentration of urea N can be used to identify dietary AA imbalance (Coma et al., 1995; Chen et al., 1999a, 1999b). Plasma albumin accounts for 60% of total plasma proteins and is a major contributor for maintaining plasma osmotic pressure for assisting with transporting lipids and steroid hormones (Matejtschuk et al., 2000; Regmi et al., 2018). The level of plasma albumin is also a good indicator of the effectiveness of dietary protein utilization, as well as of the protein synthesis capacity of the liver (Lowrey et al., 1962; Mahdavi et al., 2012; Regmi et al., 2018). The plasma concentrations of glucose, triglycerides, and cholesterol reflect the metabolic status of energy and lipids (Wu, 2018).

Shen et al. (2014) reported that pigs fed L-Met supplemented diets had lower concentrations of plasma urea N than the pigs fed DL-Met

**Table 6.** The concentrations of free AAs in the blood plasma of growing pigs before being fed two experimental diets[a]

| | Dietary treatment | | |
|---|---|---|---|
| AA, nmol/mL[b] | Diet 1 | Diet 2 | *P* value[c] |
| Lysine | 61.8 ± 13.95 | 75.3 ± 30.00 | 0.222 |
| Methionine | 50.7 ± 11.38 | 43.6 ± 15.74 | 0.259 |
| Leucine | 153.7 ± 40.18 | 126.7 ± 43.26 | 0.166 |
| Histidine | 107.9 ± 65.47 | 118.0 ± 43.17 | 0.689 |
| Phenylalanine | 80.3 ± 13.44 | 75.8 ± 18.74 | 0.548 |
| Isoleucine | 90.5 ± 28.60 | 77.6 ± 28.8 | 0.326 |
| Threonine | 283.7 ± 113.07 | 254.4 ± 119.78 | 0.580 |
| Valine | 153.4 ± 44.42 | 129.6 ± 41.15 | 0.230 |
| Tryptophan | 46.0 ± 14.04 | 45.9 ± 15.92 | 0.983 |
| Arginine | 164.5 ± 33.00 | 153.2 ± 40.06 | 0.502 |
| Citrulline | 79.3 ± 29.61 | 91.6 ± 89.11 | 0.684 |
| Alanine | 542.6 ± 148.68 | 428.3 ± 162.33 | 0.118 |
| Glutamate | 233.2 ± 149.57 | 161.1 ± 94.16 | 0.213 |
| Glycine | 675.4 ± 131.07 | 657.6 ± 295.51 | 0.864 |
| Asparagine | 94.4 ± 26.63 | 77.8 ± 30.93 | 0.213 |
| Aspartate | 24.2 ± 10.60 | 17.8 ± 7.18 | 0.133 |
| β-Alanine | 29.0 ± 25.61 | 36.7 ± 38.86 | 0.610 |
| Glutamine | 1082.5 ± 299.35 | 945.1 ± 288.42 | 0.310 |
| Ornithine | 134.0 ± 28.03 | 125.4 ± 39.91 | 0.575 |
| Serine | 140.5 ± 37.66 | 128.5 ± 49.86 | 0.552 |
| Taurine | 102.6 ± 48.16 | 86.1 ± 33.67 | 0.386 |
| Tyrosine | 96.4 ± 22.73 | 81.1 ± 18.91 | 0.119 |

[a]Diet 1 = a L-methionine supplemented diet; diet 2 = a DL-methionine supplemented diet. The calculated dietary standardized ileal digestible methionine contents in both diets 1 and 2 were 0.37% (as-fed basis).

[b]Each value is a mean ± SD ($n$ = 10).

[c]*P* values were obtained from Student's *t*-test.

supplemented diets on day 10 but not on day 20. However, Tian et al. (2016) reported that pigs fed L-Met supplemented diets had similar plasma concentrations of albumin, total protein, and plasma urea N when compared with pigs fed with DL-Met supplemented diets. Results of the present experiment are in agreement with the data from Tian et al. (2016).

In this study, pigs fed the L-Met supplemented diet had concentrations of Met and all other AA as well as taurine (an end product of Met metabolism) that were not different form that of pigs fed the DL-Met. These results agree with Tian et al. (2016), who reported that L-Met supplementation did not result in differences in plasma AA concentrations compared with pigs fed the DL-Met supplemented diets, which indicated that DL-Met and L-Met provide quantities of L-Met that are not different. Results obtained from this study indicate that the effects of L-Met and DL-Met on AA, lipid, and energy metabolism are not different.

### *Myogenic Gene Expression*

Myogenesis, the process of muscle growth, is regulated by a broad spectrum of cell signaling molecules (Bentzinger et al., 2012). Among the hierarchical interactions between those molecules, the families of myogenic regulatory factors (MRF) and myocyte enhancer factor 2 (MEF2) for the transcription factor mediated regulation are key regulators of muscle growth and development and have been a focus of many previous studies in humans and animals (Townley-Tilson et al., 2010; Wen et al., 2014). However, little is known about the effects of Met on the expressions of these factors in swine.

The MEF2 family comprise *Mef2A*, *Mef2B*, *Mef2C*, and *Mef2D*, whereas the MRF family comprise myogenic differentiation 1 (*MyoD1*), myogenic factor 5 (*Myf5*), myogenin (*MyoG*), and muscle regulatory factor 4 (*Mrf4*, a.k.a. *Myf6*). Genes in the MRF family are highly conserved and are collectively expressed in the skeletal muscle of humans (Bentzinger et al., 2012). Assisted by the MEF2 family of transcription factors (together with other general and muscle-specific factors), MRFs coordinate the activities of a host of coactivators and corepressors, resulting in a tight control of gene expression during myogenesis (Black and Olson, 1998; Berkes and Tapscott, 2005).

**Table 7.** The concentrations of free AAs in the blood plasma of growing pigs after being fed two experimental diets for 4 wk[a]

| | Dietary treatment | | |
|---|---|---|---|
| AA, nmol/mL[b] | Diet 1 | Diet 2 | *P* value[c] |
| Lysine | 203.9 ± 144.55 | 203.3 ± 104.14 | 0.992 |
| Methionine | 52.6 ± 29.55 | 47.9 ± 20.30 | 0.684 |
| Leucine | 182.0 ± 85.5 | 215.1 ± 90.28 | 0.410 |
| Histidine | 105.6 ± 37.63 | 106.6 ± 54.70 | 0.964 |
| Phenylalanine | 67.8 ± 34.83 | 75.8 ± 30.70 | 0.591 |
| Isoleucine | 124.0 ± 77.00 | 141.4 ± 65.35 | 0.592 |
| Threonine | 264.9 ± 174.83 | 293.9 ± 144.97 | 0.691 |
| Valine | 311.5 ± 202.19 | 348.0 ± 182.09 | 0.676 |
| Tryptophan | 77.9 ± 36.97 | 88.7 ± 34.05 | 0.505 |
| Arginine | 153.5 ± 88.82 | 162.3 ± 55.42 | 0.795 |
| Citrulline | 63.7 ± 32.94 | 56.4 ± 21.26 | 0.555 |
| Alanine | 558.7 ± 332.46 | 578.2 ± 287.69 | 0.890 |
| Glutamate | 298.6 ± 229.07 | 259.9 ± 166.02 | 0.670 |
| Glycine | 969.6 ± 345.51 | 1028.4 ± 324.70 | 0.699 |
| Asparagine | 93.4 ± 64.70 | 89.4 ± 38.07 | 0.867 |
| Aspartate | 33.8 ± 28.98 | 27.1 ± 11.42 | 0.506 |
| β-Alanine | 40.2 ± 30.23 | 36.4 ± 29.41 | 0.778 |
| Gluamine | 1147.6 ± 610.83 | 1145.6 ± 443.26 | 0.993 |
| Ornithine | 129.2 ± 58.12 | 140.1 ± 54.79 | 0.670 |
| Serine | 157.5 ± 71.10 | 178.6 ± 73.07 | 0.523 |
| Taurine | 103.0 ± 48.11 | 133.4 ± 52.98 | 0.196 |
| Tyrosine | 103.7 ± 51.3 | 118.1 ± 45.68 | 0.518 |

[a]Diet 1 = a L-methionine supplemented diet; diet 2 = a DL-methionine supplemented diet. The calculated dietary standardized ileal digestible methionine contents in both diets 1 and 2 were 0.37% (as-fed basis).

[b]Each value is a mean ± SD ($n$ = 10).

[c]*P* values were obtained from Student's *t*-test.

**Table 8.** The mRNA expression of selected myogenesis genes by the pigs fed a L- or DL-methionine supplemented diet before and after the 4-wk feeding trial[a]

| | | Dietary treatment | | |
|---|---|---|---|---|
| Gene name | Gene symbol | Diet 1 | Diet 2 | *P* value[b] |
| Before the feeding trial (day 0) | | | | |
| Myogenic differentiation 1 | *MYOD1* | 0.79 ± 0.21 | 1.17 ± 0.73 | 0.140 |
| Myogenin | *MYOG* | 1.09 ± 0.42 | 1.34 ± 1.02 | 0.491 |
| Myogenic factor 5 | *MYF5* | 0.71 ± 0.18 | 0.95 ± 0.47 | 0.162 |
| Myogenic regulatory factor 4 | *MRF4* | 1.38 ± 0.48 | 1.74 ± 1.82 | 0.563 |
| Myocyte enhancer factor 2A | *MEF2A* | 4.36 ± 6.49 | 1.26 ± 1.07 | 0.193 |
| Myocyte enhancer factor 2B | *MEF2B* | 1.58 ± 0.61 | 1.58 ± 1.33 | 1.000 |
| Myocyte enhancer factor 2C | *MEF2C* | 1.14 ± 0.44 | 2.07 ± 2.72 | 0.312 |
| Myocyte enhancer factor 2D, transcript variant X1 | *MEF2D* | 1.58 ± 0.64 | 2.35 ± 3.79 | 0.562 |
| After the feeding trial (day 28) | | | | |
| Myogenic differentiation 1 | MYOD1 | 1.03 ± 0.25 | 1.08 ± 0.49 | 0.794 |
| Myogenin | MYOG | 1.22 ± 0.50 | 1.05 ± 0.98 | 0.660 |
| Myogenic factor 5 | MYF5 | 1.25 ± 0.06 | 1.12 ± 0.52 | 0.475 |
| Myogenic regulatory factor 4 | MRF4 | 1.30 ± 0.26 | 1.27 ± 1.05 | 0.940 |
| Myocyte enhancer factor 2A | MEF2A | 0.66 ± 0.22 | 0.90 ± 0.53 | 0.272 |
| Myocyte enhancer factor 2B | MEF2B | 0.53 ± 0.27 | 1.17 ± 1.19 | 0.175 |
| Myocyte enhancer factor 2C | MEF2C | 2.39 ± 0.48 | 1.69 ± 2.26 | 0.553 |
| Myocyte enhancer factor 2D, transcript variant X1 | MEF2D | 0.90 ± 0.44 | 1.12 ± 1.10 | 0.614 |

[a]Diet 1 = a L-methionine supplemented diet; diet 2 = a DL-methionine supplemented diet. The calculated dietary standardized ileal digestible methionine contents in both diets 1 and 2 were 0.37% (as-fed basis).

[b]*P* values were obtained from Student's *t*-test.

The lack of a difference in the expression of myogenesis-related genes in pigs fed the two sources of Met indicates that there is no difference between L-Met and DL-Met in terms of their effects on the muscle growth and development of growing pigs. These gene expression data together with the performance data indicate that D-Met can be efficiently converted into L-Met to provide enough bioavailable Met for pig myogenesis.

## CONCLUSIONS

Results of this experiment indicate that equal dietary supplementation of L-Met or DL-Met to a Met deficient diet results in growth performance of young pigs that is not different. Pigs fed the DL-Met supplemented diet also had a nutritional status that was not different from that of pigs fed the L-Met supplemented diet as indicated by plasma concentrations of AA that were not different. Expression of myogenesis genes by the pigs was not differently affected by the two Met sources either. With no difference in nutritional bioefficacy between L-Met and DL-Met, the selection between the two sources for feeding pigs can be exchangeable, assuming that the commercial prices for the two sources of Met are the same.

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